Supplementary Information

A Quantitative Microfluidic Angiogenesis Screen for Studying Anti-Angiogenic Therapeutic Assay

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SI Materials and Method

Computational modeling of concentration profiles.

Gradients of growth factors within the collagen scaffold were quantified by computational modeling using the coupled transient convection-diffusion and Brinkmann equations, which were solved using a commercial finite element solver, COMSOL (Burlington, MA) [1]. In computational simulations, the diffusion constant of a 40 kDa inert molecule (VEGF) in the collagen matrix and the matrix hydraulic permeability ($K=10^{-13}$ m^2) were based on the measurements described previously [2, 3], the diffusion coefficient in the medium was set to 6×10^{-11} m^2/s, the growth factor diffusion coefficient in the scaffold was defined to be 5×10^{-11} m^2/s [2]. In the device, flow-velocities were controlled from u = 0.07 μm/s (Peclet (Pe) number =0.5) to 7 μm/s (Pe=50).

Quantitative analysis of EC morphology by the treatment of bortezomib

The effects of bortezomib on EC morphology in the model were analyzed quantitatively. To analyze easily EC morphology by circularity analysis, Red expressing HUVECs were used. In day 5, the cells were fixed in 4% PFA and stained for Nuclei. Five images from each experiment were captured using a confocal microscope, and 10 random cells per image were traced using Image J. At least three experiments were performed. The circularity was calculated from the area and perimeter of the traced EC using the formula [4]:

$$Circularity=4\pi\ (Area/Perimeter^2)$$

A circularity of 1.0 indicates a perfect circle. As the circularity approaches 0.0, the shape becomes an increasingly elongated ellipse.

SI References

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[2]. Zervantonakis IK, Chung S, Sudo R, Zhang M, Charest JL, Kamm RD. Concentration gradients in microfluidic 3D matrix cell culture systems. Concentration gradients in microfluidic 3D matrix cell culture systems. *Intern J Micro-Nano Scale Transport*, 2010, **1**(1), 27-36.

[3]. Vériter S, Mergen J, Goebbels RM, Aouassar N, Grégoire C, Jordan B, Levêque P, Gallez B, Gianello P, Dufrane D. In vivo selection of biocompatible alginates for islet encapsulation and subcutaneous transplantation, *Tissue Eng Part A,* 2010, **16**(5), 1503-1513.

[4] Kasuya J, Sudo R, Mitaka T, Ikeda M, Tanishita K. Hepatic stellate cell-mediated three-dimensional hepatocyte and endothelial cell triculture model, *Tissue Eng Part A*, 2011, **17** (3-4), 361-370.

SI Figure legends

Figure S1. (A) The QMAS consists of: (1) reservoirs used for providing fresh conditioned media (VEGF-supplemented or Bortezomib-supplemented medias) into all the channels, (2) a 1st PDMS layer (cell culture layer) including microchannels for the bortezomib-supplemented media with various concentrations, endothelial cell monolayers and collagen gel-cages (channel depth= 150μm), (3) 2nd PDMS layer (manifold layer) used only during filling as a manifold layer for filling collagen gel , and (4) a large size cover slip at the bottom. (B) Photograph of the complete system.

Figure S2. (A) Schematic view of a 1st PDMS layer (cell culture layer) including microchannels for the bortezomib-supplemented media with various concentrations, endothelial cell monolayers and collagen gel-cages. (B) Schematic diagram of fluidic resistance network of the 1st PDMS layer (cell culture layer). (C) Generation of uniform flow rates in all micrhchannels during perfusion culture with hydrodynamic simulation.

Figure S3. (A) The concentration gradient profile along the length of microfluidic channel under the Peclet (Pe) number from 0.5 to 50. The concentration profile along the length of channel was formed nearly constant under the more than Pe 50. (B) The concentration gradient profile across the scaffold regions under the Peclet (Pe) number from 0.5 to 50. The linear concentration gradient across the scaffold regions was also generated more than Pe 50.

Figure S4. (A) Schematic view of a 1st PDMS layer (cell culture layer) and 2nd PDMS layer (manifold layer) for filling collagen gel. (B) Schematic diagram of fluidic resistance network of the 2nd PDMS layer (manifold layer). (C) Generation of the same volumetric flow rates in the 14 collagen gel regions using hydrodynamic simulation.

Figure S5. A set of experiments to demonstrate the capabilities of the QMAS as an angiogenesis assay in response to chemoattractant gradients. VEGF-supplemented mediums (40 ng/ml) were introduced into the VEGF injection channels (#1, #3, #5, & #7) and the control media without VEGF were injected into the VEGF injection channels (#2, #4, #6, & #7) as reference

conditions.

Figure S6. A set of experiments to mimic the *in vivo* blood vessels for treatment of cancer on the dose of logarithmic concentration of bortezomib (0, 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1}, and 1 μM). The endothelial cell monolayers on the walls of the channels were formed through the injection of endothelial cells (HUVECs) by the multiple-pipette (drug injection channels (#1 ~ #8). Secondly, VEGF (40 ng/ml)-supplemented media mimicking the growth factors (i.e. VEGF) secreted from the tumor were filled in the VEGF injection channels, and finally, media containing a range of concentrations of bortezomib (logarithmic concentrations: 0, 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1}, and 1 μM) were introduced in each reservoir all with the multiple-pipette (drug injection channels (#1 ~ #8)).

Figure S7. A set of experiments to mimic the *in vivo* blood vessels for treatment of cancer on the dose of linear concentration of bortezomib (0, 1 (10^{-3} μM), 2, 4, 6, 8, and 10 nM (10^{-2} μM)). The endothelial cell monolayers on the walls of the channels were formed through the injection of endothelial cells (HUVECs) by the multiple-pipette (drug injection channels (#1 ~ #8). Secondly, VEGF (40 ng/ml)-supplemented media mimicking the growth factors (i.e. VEGF) secreted from the tumor were filled in the VEGF injection channels, and finally, media containing a range of concentrations of bortezomib (linear concentrations: 0, 1, 2, 4, 6, 8, 10 nM) were introduced in each reservoir all with the multiple-pipette (drug injection channels (#1 ~ #8)).

Figure S8. (A) The morphology of HUVECs on the dose of logarithmic concentration of bortezomib (0, 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1}, and 1 μM). Typical cobblestone-like morphology of HUVECs was observed at the low concentrations of bortezomib (0, 10^{-5}, and 10^{-4} μM) by day 5, whereas the HUVECs showed some elongated morphological shapes without spindle-like shapes when conditioned with a dose of 10^{-3} μM. Almost all cells died when exposed to bortezomib at higher concentrations (10^{-2}, 10^{-1}, and 1 μM). (B) The morphology of HUVECs on the dose of linear concentration of bortezomib (0, 1 (10^{-3} μM), 2, 4, 6, 8, and 10 nM (10^{-2} μM)). The morphological changes in HUVECs occurred in the range of 4 nM and 6 nM bortezomib. Within the narrow range, HUVECs neither died nor detached from the surface and only showed slight changes in their structures. Almost all cells died at the dose of 8 nM and 10nM, became detached from the surface and were washed away during media changes.

Figure S9. (A) The morphology of HUVECs at the concentration of 10^{-2} μM. The morphological transition to a spindle-like shape was discernable from day 1, and dead cells, which were detached from the cell surface, were observed from days 2 to 5. Consequently, bortezomib-induced endothelial cell death was preceded by a series of morphological changes that develop over an extended period. (B) Qualitative analysis of ECs morphology by circularity analysis. Red expressing HUVECs were used. Five images from each experiment were captured using a confocal microscope, and 10 random cells per image were traced using Image J. A circularity of 1.0 indicates a perfect circle. As the circularity approaches 0.0, the shape becomes an increasingly elongated ellipse.

Figure S10. The projected images on the dose of logarithmic concentration of bortezomib (0, 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1}, and 1 μM)**.** Confocal microscopy revealed the growth of circular lumen-like structures from the HUVECs monolayer into the collagen scaffold in response to VEGF at lower bortezomib concentrations (0, 10^{-5}, 10^{-4}, & 10^{-3} μM), but no morphogenesis for doses of

10^{-2}, 10^{-1}, and 1 μM was evident in immunostained and confocal microscopy images. (B) The projected images at the concentration of 10^{-2} μM. HUVECs migrated toward the 3D hydrogel scaffolds in response to VEGF for 2 days, but those migrated cells regressed after 3 days.